INDOCYANINE GREEN (ICG) COMPOSITIONS AND RELATED METHODS OF USE

RELATED APPLICATIONS

[0001] This application is a continuation-in-part of co-pending U.S. patent application 09/393,456, filed September 10, 1999, which is incorporated by reference.

FIELD OF THE INVENTION

[0002] The present invention generally concerns indocyanine green compositions useful in the diagnosis of organ function and disease in animals, e.g., humans.

BACKGROUND OF THE INVENTION

[0003] Indocyanine green (ICG) is a well-known fluorsecent dye. The dye is presently marketed by Akorn, Inc. (Buffalo Grove, Illinios) under the trademark IC GREEN™. ICG is presently supplied as a lyophilized powder (25 mg) for reconstitution with 5 ml sterile water for injection (WFI). The reconstituted ICG composition (at 5 mg/ml) should be used within 10 hours, with any unused portion being discarded.

[0004] The U.S. Food and Drug Adminstration has approved this dye as an injectable drug for use in determining hepatic function, cadiac output and liver blood flow, as well as for opthalmic angiography. In opthalimic angiography, the ICG is excited to fluorescence by radiation, permitting angiograms of the opthalmic vasculature to be obtained.

[0005] Although currently available aqueous ICG compositions provide adequate levels of ICG for use in the approved indications, the solubility of ICG in WFI decreases as the concentration exceeds 5 mg/ml. Thus, a need exists for an aqueous ICG composition that exhibits beneficial properties exceeding those possessed by the currently approved compositions, particularly with respect to enhancements in ICG concentration and stability.

SUMMARY OF THE INVENTION

[0006] In one aspect, the present invention provides an ICG composition that exhibits enhanced stability, as well as enhanced ICG concentration, as compared to presently available ICG products. The composition comprises an aqueous ICG composition comprising ICG at a concentration of at least about 10 mg/ml and an aqueous diluent, wherein the composition is stable for at least 24 hours. In a related aspect, the invention provides a stable ICG liposomal formulation.

[0007] Among others, the inventive compositions provide enhanced angiographic resolution relative to that provided by the currently approved ICG composition, as well as certain economic advantages. For example, the same ICG composition may be administered to a given patient over a course of several days, as opposed to preparing a fresh composition prior to each administration.

[0008] Other aspects of the present invention include methods for using the inventive compositions. These methods include the presently approved uses, and the diagnosis and treatment of age-related macular degeneration (ARMD)-related choroidal neovascularization (CNV), tumors, and other undesirable lesions fed by newly-formed and exisitng blood vessels. Illustrative techniques useful in these methods include angiography, dye-enhanced photocoagulation of blood vessels, photodynamic therapy (PDT), and combinations thereof.

[0009] When administered in connection with angiography, the relatively high concentration ICG formulations of the present invention permit more rapid and accurate identification of vessels, e.g., vessels that feed blood to a lesion. When treatment of the feeder vessel or lesion, such as a tumor or CNV, via dye-enhanced photocoagulation is desired, the inventive compositions provide faster and more permanent occulsion of these abnormalities. Further, less energy is required to occlude the abnormalities as opposed to the energy required using conventional ICG compositions.

[0010] The inventive ICG compositions have been administered to rats and found to be safe when measured by hematology, clinical chemistry, histology and pathology of tissue samples.

[0011] These and other features and advantages of the present invention will become apparent upon review of the following figures and detailed description of the preferred embodiments of the present invention.

DESCRIPTION OF THE DRAWINGS

[0012] FIGURE 1 is a graph demonstrating the excitation fluorescence spectra between 500 and 820 nm of a preferred ICG formulation of the present invention using Water For Injection (WFI) as the water source.

[0013] FIG. 2 is an emission spectra of a preferred ICG formulation of the present invention.

[0014] FIG. 3 is a graph representing the stability of two ICG formulations, one comprising ICG and WFI and the other a preferred ICG formulation of the present invention.

[0015] FIG. 4 is X-ray diffraction data for a lyophilized form of ICG.

[0016] FIG. 5 is X-ray diffraction data for a crystalline form of ICG.

DETAILED DESCRIPTION OF THE INVENTION

[0017] In one aspect, the present invention provides aqueous ICG compositions that provide for a relatively higher ICG concentration and greater stability relative to the currently approved product. In a preferred aspect, the inventive formulations provide at least 10 times, more preferably at least 15 times, the present ICG concentration, while also possessing a stability of at least 10, and more preferably at least 15, times relative to the currently-approved ICG product.

[0018] The inventive compositions comprise ICG at a concentration of at least 10 mg/ml and an aqueous diluent, wherien the ICG is stable for at least 24 hours. Advantageously, the ICG is stable in the aqueous compostion for at least 48 hours, preferably for at least 3 days, more preferably for at least 5 days, and most preferably for at least 7 days, despite the ICG being present at relatively high concentrations.

[0019] The amount of ICG that may benefit from the stability provided by the inventive diluent varies widely, from about 1 mg/mL up to about 100 mg/mL. Of course, benefits of the present invention will be obtained at ICG concentrations exceeding that which is presently approved. Advantageously, then, enhanced solubility and/or stability is desired, and should be noticed, at ICG concentrations of at least about 10 mg/ml, 20mg/ml, 25 mg/mL, 50 mg/mL, 75 mg/mL, and up to at least about 100 mg/mL, and at ranges therebetween.

[0020] The ICG used in the inventive composition may be provided in any suitable form, but is most commonly provided in a sterile lyophilizate. When provided as a lyophilizate, the ICG is reconstituted prior to adminstration by use of the aqueous diluent described herein. To effect the reconstitution, water, in the form of sterile WFI, may be introduced into a vial holding the ICG, with the aqueous diluent being added thereafter. Alternatively, the WFI and aqueous diluent may be added in reverse order. Preferably, however, the aqueous diluent is in a vial separate from the ICG vial, with the aqueous diluent being introduced into the ICG-containing vial in an amount sufficient to provide the desired final ICG concentration, more preferably without the need for additional dilution to obtain the desired final ICG concentration.

[0021] The ICG and diluent may also be packaged together, e.g., as a kit, or in a dual chamber configuration, such as a pre-loaded dual chamber syringe or vial. Such syringes and vials maintain separation between the ICG and diluent, but permit mixing upon activation, prior to administration.

[0022] The aqueous diluent advantageously comprises a solubilizer and alcohol, with water (preferably sterile WFI) being added to reach the desired dilution. Desirably, the solubilizer is provided, per ml of diluent, at about 0.5 to about 5 mg, with the alcohol provided at about 50 to about 150 mg on the same basis.

[0023] While not wishing to be bound to any particular theory, the solubilizer is theorized to assist in solubilizing the ICG in the aqueous diluent, while also enhancing the stability of the final ICG composition. Suitable solubilizers for use in the inventive composition include surface active agents (also referred to as surfactants) and cosolvents (e.g., polyethylene glycol). Surfactants are preferred, with liquid (at 25°C) nonionic surfactants being most preferred, e.g., Tweens, such as polysorbate 80. In either case, the solubilizer is advantageously a liquid (no more than about 5 cp viscosity, preferably no more than about 3 cp, and most preferably about 1 cp, at 25°C), e.g., polyethylene glycol

having a molecular weight of less than about 800, and more preferably less than about 500. This will assist in promoting solvation of the ICG in the diluent, and enhance the ability of the aqueous ICG composition to be injected into the bloodstream.

[0024] The solubilizer should be present in the ICG composition in an amount sufficient to enhance the solubility of the ICG in the composition relative to the same composition without the solubilizer, and also relative to the solubility of the presently approved ICG compositions. However, is was unexpectedly found that the inclusion of excessive levels of solubilizer in these relatively concentrated compositions adversely affected the composition stability. Desirably, then, the amount of solubilizer is limited to that which provides the aqueous composition with a stablizing effect, typically no more than about 7 mg/ml of the aqueous diluent, and advantageously no more than about 5 mg/ml. More preferably, the solubilizer is present at from about 0.1 mg/ml, and is more preferably present at from about 0.25 mg/ml to about 5 mg/ml of the aqueous diluent.

[0025] Generally, the ratio of solubilizer to ICG in the aqueous ICG composition, on an absolute weight basis, may range from about 0.1:100 to about 7:10, advantageously from about 0.2:100 to about 5:75, and preferably from about 0.2:75 to about 3:75.

[0026] A lower alkyl alcohol is also included in the composition, in part due to its ability to enhance the solubility of ICG in the diluent, as well as enhance the fluorescence of ICG. While a variety of pharmaceutically-acceptable alcohols may be used, lower alkanols (C_2-C_6 alcohols), diols and triols are advantageously utilized, e.g., ethyl alcohol, glycerine, propylene glycol and mixtures thereof. Ethyl alcohol is preferred, due to its low cost, pharmaceutical-acceptability and wide availability.

[0027] Generally, sufficient alcohol should be included in the diluent to provide enhanced solubility and/or fluorescence of ICG relative to the same composition without alcohol, and also relative to the presently approved ICG composition. Generally, the ratio of alcohol to ICG on a weight basis may range from about 1:0.25 to about 1:4, is preferably about 1:0.5 to about 1:3, and most preferably about 1:1 to about 1:2.5. Optimally, the ratio is about 1:2. On a weight percentage basis, the alcohol should be present in the aqueous diluent from about 25 mg/ml to about 250 mg/ml of the diluent, advantageously from about 50 mg/ml to about 200 mg/ml, and preferably from about 75 to about 125, to about 150 mg/ml.

[0028] The amount of alcohol in the diluent is further preferably greater than the amount of solubilizer. The former is preferably present in at least 50 wt.% excess, up to and including about 200 wt.% excess, relative to the solubilizer. More preferably, the alcohol is at least 75 wt.%, up to and including 150 wt.%, and most preferably about 85 wt.% to about 125 wt.%, relative to the weight of solubilzer.

[0029] The diluent further desirably includes polyvinyl pyrrolidone (PVP). This component is believed to contribute to the stability of the composition, and is therefore

included in a stability-enhancing amount. Generally, this component may be present in the aqueous composition, per ml of diluent, from about 10 to about 100 mg, advantageously from about 25 to about 75 mg, and preferably from about 40 to about 60 mg. As PVP is a polymer, and available at a variety of molecular weights, it is desirable to utilize a relatively low molecular weight PVP (less than about 30,000, preferably less than about 15,000) to assist in maintaining the viscosity of the aqueous composition at a relatively low level.

[0030] Preferably, but optionally, the diluent includes an antimcrobial agent. This component assists in maintaining the sterility of the diluent during storage. While any number of pharmaceutically-acceptable antimicrobials may be used, an antimicrobial that does not adversely affect the solubility and other beneficial properties of the aqueous ICG composition is preferred. Illustrative of such antimicrobials are those containing alcohol functionalities, such as benzoyl alcohol.

[0031] The amount of antimicrobial agent is advantageously that which provides the foregoing advantages, including imparting antimicrobial activity to the diluent during storage and the aqueous ICG composition. Preferably, the antimicrobial is provided, per ml of diluent, at from about 5 mg to about 10 mg per ml of diluent.

[0032] The water included in the inventive composition is preferably sterilized, e.g., WFI. The amount of water used in the diluent is that required to provide the desired level of ICG concentration in the ICG compositions, as well as the desired weight percentages of the other diluent components.

[0033] As an alternative, one or more of the diluent components may be lyophilized with the ICG. For example, and if included, a pH adjusting agent and/or antimicroial agent may be lyophilized with the ICG and retained within the ICG vial until reconstitution with the remaining components of the diluent. Other such combinations of diluent components and ICG are possible, depending on the ability of each particular diluent component to sucessfully undergo lyophilization with the ICG.

[0034] A related aspect of the present invention is a liposomal ICG formulation. In this formulation, the ICG is provided as a lyophilizate which, upon reconsitution with a diluent comprising water (e.g., WFI, saline, and liposome-forming components), provides for enacapsulation of the ICG in liposomes. Alternatively, the liposome-forming components may be included with the ICG in the lyophilizate composition. After reconstitution, the ICG is desirably present at a concentration of about 0.5 to about 3 mg/ml of the formulation, and more desirably from about 1 to about 2 mg/ml. When reconstituted, the liposomal formulation may comprise from about 1 to about 100 mg ICG.

[0035] The liposome-forming components may be selected from those which are pharmaceutically-acceptable. Illustrative components include dl-alpha tocopheryl acetate

(about 0.1 to about 1mg), cholesterol (about 5 to about 50mg), egg phosphatidylcholine (about 10 to about 100mg) and tertiary butyl alcohol (about 0.1 to about 10 ml), on a per ml basis.

[0036] The inventive composition provides the further advantage of relatively low viscosity, rendering it suitable for parenteral administration. More specifically, the viscosity of the diluent, after one month of storage at ambient (25°C) temperature, will advantageosly remain less than about 5 centipoise, and preferably less than about 3 cp. The aqueous ICG composition desirably remains less than about 5 cp, preferably less than about 3 cp, and most preferably about 1 cp, up to one week after reconstitution.

[0037] A pH adjustment of the reconstituted compositon to between about 6 to about 8 may optionally be completed using effective amounts of any of a number of pharmaceutically-acceptable acids, bases and/or buffer systems. Preferably, an acid and/or base is used in an effective amount, e.g., HCl, NaOH, to adjust the pH of the reconstituted composition to its preferred level of about between about 6.5 and 7.5, and more preferably about 7.

[0038] Related aspects of the present invention concern methods of using the compositions herein described. These methods are claimed and described as a series of diagnostic and/or treatment steps. It should be understood that these methods and associated steps may be performed in any logical order. Moreover, the methods may be performed alone, or in conjunction with other diagnostic procedures and treatments administered before, during or after such methods and steps set forth herein without departing from the scope and spirit of the present invention. It is further contemplated that the term animals, as used herein, includes, but is not limited to, humans.

[0039] As used herein, stability may be described in terms of a drop in potency of the ICG compositions. For example, the drop in potentcy is desirably less than about 10%, preferably less than about 7%, more preferably less than about 5%, and most preferably less than about 2%, after one or more of the aforementioned time periods (e.g., 24 hours, 48 hours, 3 days, one week, 2 weeks, 3 weeks and 4 weeks, etc.), when stored in a 25°C environment, and under refrigerated conditions (i.e., between 4-8°C). Preferably, the aqueous ICG composition will also possess one or more of the following attributes: no visual precipitate (naked eye, preferably under 25X, and more preferably under 50X examination), no color change (as viewed by the naked eye relative to a freshly prepared equivalent ICG composition), no loss in sterility, and/or no greater than about 2 wt.%, and more preferably no greater than about 1 wt.% degradation product, over one or more of the aforementioned time periods. A determination of relative composition potency may be obtained using HPLC. Sterility may be measured using any one of several tests therefor sanctioned by the U.S. Food and Drug Administration.

[0040] The aforementioned stability parameters may also be used in the evaluation of the inventive aqueous ICG compositions under accelerated testing. For example, when the compositions are placed in a 40°C environment, they desirably remain stable for at least 4 hours, preferably for at least 8 hours, and more preferably for at least 12 hours.

[0041] The ICG and diluent may be packaged in any suitable manner, e.g., vials made of glass, plastic or other pharmaceutically-acceptable materials. The diluent and aqueous ICG compositions are desirably protected from exposure to light in a green, amber or opaque container. Preferably, the ICG and diluent are packaged in a multi-chambered vessel which segregates the ICG from the diluent until the aqueous ICG composition is required for therapy. Examples of suitable muti-chambered vessels include a dual-chamber by-pass syringe and a dual chambered vial which enables mixing of the ICG ad diluent as desired.

[0042] A further aspect of the present invention contemplates methods for using the foregoing ICG compositions. These methods are generally diagnostic and/or therapeutic in nature. In one preferred aspect, the invention provides for the same diagnostic methods as those currently approved for ICG, but use the inventive ICG compositions described herein as the ICG source instead of the standard aqueous ICG formulation. For example, the inventive compositions may be used in obtaining angiographic images in association with the diagnosis of any disease or condition in which angiography is a useful diagnostic tool. Such diagnostic procedures are well known, and continue to be developed, e.g., the diagnosis of CNV.

[0043] Other method contemplated by the present invention concern the diagnosis and/or treatment of conditions, particularly lesions, the treatment aspect using the inventive composition in dye-enhanced photocoagulation. Generally, in this method, radiation of a certain wavelength (based upon the dye used) is applied onto an undesired portion of a dye-carrying blood vessel, e.g., a vessel that carries, or feeds, blood to the lesion. The radiation, once a wavelength is applied that will "excite" the dye, causes the temperature of the dye to increase upon absorption of the radiation. While not desiring to be bound to any particular theory, as the dye temperature increases, the temperature of the surrounding blood and vessel tissue also increases. This increase in temperature hastens the rate at which blood clots in and adjacent that portion of the vessel onto which the radiation is applied. This clotting, in turn, leads to partial, or preferably complete, obstruction of the vessel in or adjacent the portion of the vessel onto which the radiation was applied. This obstruction will, in many instances, provide for subsequent reduction in the lesion. Alternatively, or in connection with this therapy, the lesion itself may be irradiated in the presence of the dye.

[0044] It is well known that the peak absorption and emission of ICG lies in the range of 800-850 nm. Thus, a light source emitting such wavelength should be used when

obtaining angiographic images during a diagnostic procedure, as well as during any therapeutic procedure (with power being modulated accordingly).

[0045] It should be appreciated that in connection with the inventive methods (e.g., diagnosis of lesions such as CNV, tumors and abnormal vasculature), the amount of ICG administered should be sufficient to permit the dye to fluoresce when radiation at the appropriate wavelength is applied, thereby providing useful angiographic images. The same standard is applicable to the therapeutic methods; sufficient dye should be utilized to enable the desired treatment. This information may be readily determined by those skilled in the art, and should be at least that concentration currently accepted for use in ophthalmic angiography, e.g., for diagnosis, 2 ml of a 20 mg/mL ICG solution (IC-GREEN™). Of course, the relatively higher dye concentrations described herein may advantageously be used in any of these diagnostic and treatment methods.

[0046] Any suitable source of radiation that causes the particular dye to fluoresce as it flows through the vessels of interest may be used in the present methods. The type and amount of energy applied to the blood vessels of interest must be sufficient to cause the fluorescent dye present in these blood vessels to fluoresce. The energy applied must be within the limits of the maximum flux density or irradiance which can be applied to the blood vessels of interest within a particular time span without causing excessive damage to the normal surrounding tissue. The longer the duration of exposure to the energy source, the lower the allowable level of irradiance. The particular energy source and amount of energy applied will depend upon the type of fluorescent dye administered to the subject.

[0047] The radiation used in the methods described herein is preferably applied using a laser, and, most preferably, using a pulsed laser. The pulsing of the laser provides the advantage of generating a greater number of photons for image formation in the shortest time interval. Various devices, preferably fundus cameras for ophthalmic diagnoses and therapies, can be adapted for providing an appropriate level and type of radiation in accordance with the teachings provided herein. The latter include, for example, those described in U.S. Patents 5,279,298, 5,394,199 and 5,400,791. Preferably, a fundus camera having two sources of radiation (e.g., lasers) is provided. Using such a camera, one laser can be used to irradiate the general area of interest so any ocular vessels requiring treatment can be identified, while the second laser can be used almost immediately upon identification of the vessel to be treated to hasten the coagulation of the blood therein, i.e., dye-enhanced photocoagulation. The ability to aim the treatment laser using the identical view used to obtain the angiograms is a significant advantage. Further, the ability to complete the diagnosis and treatment steps within minutes, e.g., advantageously in less than about 30 and preferably less than about 15 minutes, lessens patient trauma and increases overall treatment efficiency.

[0048] The inventive methods further contemplate the administration of the inventive

composition in order to permit visualization of vessels at locations other than in the eye. Generally, angiograms of blood vessels and other abnormalities associated with blood vessels may be obtained at any location in an animal in which readable angiographic images can be obtained. For example, hollow organs and body cavities may be subjected to the inventive methods, e.g., the interior wall of the bladder, stomach, lung, gastrointestinal tract, bladder, pancreas, gall bladder, sinus, liver, kidney, heart, cervix, ovary, prostate, stomach, trachea, skin or colon may be explored, as well as the exterior walls of those organs, and the brain. This permits the diagnosis (including, e.g., the monitoring of prior treatments or of prior-diagnosed conditions) and treatment of lesions, e.g., abnormal blood vessels, such as aneurysms, ruptured blood vessels, as well as the diagnosis and treatment of tumors associated with those and other body cavity tissues.

[0049] An endoscope may advantageously be used to obtain the previously mentioned angiograms. The endoscope would be inserted into the body and positioned adjacent the area of interest. A first instrument would be used with the endoscope to provide radiation at an appropriate wavelength, e.g., a laser optic cable, to cause the ICG dye within the subject vessels to fluoresce so an angiogram can be obtained. Similarly, a second instrument would be used with the endoscope that would permit an angiographic image of the fluorescing ICG dye within the vessels to be obtained. For example, an optical device connected to a CCD camera, such as those used to perform a colonoscopy and other invasive procedures that permit a physician to view the interior of a body cavity, presently exists, and such technology may be readily adapted for use in conjunction with the endoscopic procedures of the present invention.

[0050] After injection of the dye composition, and flow of the composition through the region expected to be afflicted, an angiogram would then be obtained using what are referred to herein as the first and second instruments, and any abnormal vessels detected thereby treated, using the procedures described previously for diagnosis and treatment.

[0051] In the context of the present invention, the term "body cavity" includes any cavity that permits the introduction of an endoscope or other instrument that permits the use of appropriate radiation and imaging equipment required to obtain an angiogram. Illustrative of body tissues associated with suitable cavities are the eye, lung, gastrointestinal tract, bladder, pancreas, gall bladder, sinus, heart, cervix, brain, trachea, ovaries, prostate, stomach and skin.

[0052] Treatment is preferably effected by applying radiation upstream of the lesion, e.g., upstream of the ruptured blood vessel, the vessel feeding the tumor, or adjacent and upstream of the abnormal blood vessels, after administration of the dye composition. The radiation is desirably applied as the dye bolus first enters the vessel to be treated, whereby the flow of blood through the vessel is reduced. Permitting the ICG to circulate within the body for permits the ICG to stain the walls of those tissues that are contacted by the ICG.

This may result in undesired portions of the tissue being treated. While not desiring to be bound to any particular theory, when radiation is applied, the temperature of any liquid adjacent the ICG dye receiving the radiation is raised, and the blood clotting is hastened, thereby reducing, e.g., partially or completely preventing, the flow of blood through the vessel. Varicose veins may also be treated using the aforementioned treatment methods.

[0053] When the treatment of a tumor, advantageously a solid tumor, is undertaken, the method of the present invention is preferably used in combination with other treatment agents. For example, therapeutically-effective amounts of chemotherapeutic agents, such as cisplatin, carboplatin, doxorubicin, paclitaxel, taxotere, methotrexate, fluorouracil, camptothecin, cyclophosphamide and mixtures thereof, may be administered, as well as therapeutically-effective amounts of anti-angiogenesis agents, either alone or in combination, may be administered. The identity of suitable anti-tumor and anti-angiogenesis agents and associated dosage regimens are well known, and as such will not be repeated herein. The timing of administration of these agents may occur at any time so long as the administration does not interfere with the treatment method of the present invention. Advantageously, however, the agents may be administered in combination with the dye-enhanced photocoagulation treatment methods described herein. For example, the agents can be administered immediately after dye-enhanced photocoagulation of tumor feeder vessels, and preferably are injected directly into the tumor. This provides several advantages including the reduction of trauma to the patient because multiple treatment agents are administered in a single procedure, the chemotherapeutic and anti-angiogenesis agents are delivered directly to the tumor thereby limiting the exposure of healthy tissue to these toxic agents (as would be the case using conventional IV administration), and conventional radiation can be narrowly focused on the tumor itself, as opposed to conventional methods that irradiate an area surrounding the tumor.

[0054] Conventional radiation treatment, mentioned previously, surgical intervention, and photodynamic therapy (PDT, the latter using the inventive ICG compositions, under conditions which produce, as presently theorized, the production of singlet oxygen which damages the targeted tissue) may also be used individually or in combination, before, after and in some cases, if feasible, during, the diagnostic and/or treatment methods of the present invention have been used. Preferably, PDT is applied after the dye-enhanced photocoagulation therapy described herein, and more preferably without further administration of ICG. If a need for additional ICG is indicated, however, the original and additional ICG is advantageously obtained from the same source (e.g., vial).

[0055] When diagnosis of the tumor is made in accordance with the angiogram methodology of the present invention, the location and boundaries of the tumor may be determined with a high degree of precision, without resort to the use of more harmful diagnostic procedures, e.g., X-rays. The precision provided by the present invention permits

the treatment agents described previously to be more efficient because they are applied with a high degree of precision onto just the tumor itself, as compared to conventional methods, e.g., systemic administration of chemotherapeutic agents and application of radiation, which are applied over a more general area. This precise focus, in turn, lessens trauma to the subject by minimizing the side effects of these toxic agents.

[0056] The following examples are illustrative of preferred embodiments of the invention, and should not be considered as limiting the invention as defined by the appended claims in any respect.

COMPARATIVE EXAMPLE A

[0057] Baseline data concerning a commercial ICG formulation was obtained as follows:

Formulation	Per ml	Total
Indocyanine Green	5 mg	5 g
WFI	1 ml	1000 ml

[0058] 5 g ICG was added to 1000 ml of WFI and mixed in a suitable container until the ICG was completely dissolved. The resulting solution was sterilized by filtration through a 0.2 micron filter. 5 ml of the sterile solution was introduced into sterile vials and lyophilized. The vials were then stoppered, each including 25 mg ICG. The lyophilized formulation was found to be stable for a minimum of 2 years.

[0059] The sterile, lyophilized 25 mg of ICG in a vial prepared by the procedure described above was reconstituted with 5 ml of sterile WFI to provide a 5 mg/ml solution. The reconstituted solution was stable for about 10 hours when stored in a 25°C environment (e.g., no visible precipitates, decline in potency was less than about 10%, no color change, and degradation products were less than 2 wt.%).

[0060] The maximum concentration of ICG in WFI was found to be about 25 mg/ml. When formulated at an ICG concentration of 50 mg/ml to 75 mg/ml using WFI as the lone diluent, the resulting composition had a paste-like consistency.

EXAMPLE 1

[0061] This is an example of a preferred formulation of the present invention, which provides both relatively high ICG concentration and stability compared to the commercially available ICG formulation, with a neutral pH.

Formulation	Per ml	Total
Indocyanine Green	10mg	10 g
WFI	1 ml	1000 ml

[0062] 10 g of ICG was added to 1000 ml WFI and mixed in a suitable container until the ICG was completely dissolved. The solution was then sterilized through filtration using a 0.2 micron filter. 5 ml of the sterile solution was introduced into a series of sterile vials and lyophilized. The vials were then stoppered, each including 50 mg ICG. The

lyophilized formulation was found to be stable for at least 2 years.

[0063] A diluent for reconstitution of the lyophilized ICG was prepared as follows:

Formulation	Per ml	Total
Polyvinyl pyrrolidone	50 mg	50 g
Ethanol	100 mg	100 g
Polysorbate 80	2 mg	2 g
Benzyl Alcohol	10mg	10g
Sodium Hydroxide, qs pH	7.0	7.0
Water for Injection, qs ad	1 ml	1000 ml

[0064] The diluent was prepared by mixing 100 g ethanol with 10 g benzyl alcohol in a suitable container. 2 g polysorbate 80 was then added, also via mixing. 50 g polyvinyl pyrrolidone was then added and dissolved therein. Sufficient sodium hydroxide solution was added to adjust the pH to 7.0. WFI was added to bring the solution to 1000 ml. The solution was then filtered through a 0.2 micron filter, and 5 ml of the solution was introduced into a series of sterile vials. The formulation was found to be stable for at least 7 days when stored in a 25°C environment.

[0065] Sterile lyophilized 50 mg ICG was reconstituted with the aforedescribed diluent to provide a 50 mg/ml ICG solution. The reconstituted solution was found to be stable for at least 7 days when stored in a 25°C environment, demonstrating superiority over the present commercial ICG formulation described in Comparative Example A. Specifically, with respect to an ICG concentration of 5 mg/ml, the 50 mg/ml inventive formulation was about 10 times more concentrated. Surprisingly, and despite the increase in concentration, the stability of the latter was about 17 times that of the former.

EXAMPLE 2

[0066] This is a further example of a preferred formulation of the present invention, which provides a relatively higher ICG concentration and similar stability relative to the ICG formulation described in Example 1.

Formulation	Per ml	Total
Indocyanine Green	15 mg	15 g
Water for Injection, qs ad	1 ml	1000 ml

[0067] 15 g of ICG was added to 1000 ml WFI and mixed in a suitable container until the ICG was completely dissolved. The solution was then sterilized through filtration using a 0.2 micron filter. 5 ml of the sterile solution was introduced into a series of sterile vials and lyophilized. The vials were then stoppered, each including 75 mg ICG. The lyophilized formulation was found to be stable for at least 2 years.

[0068] A sterile lyophilized 75 mg ICG vial was reconstituted with a vial of the diluent described in Example 1 to provide a 75 mg/ml ICG solution. The reconstituted solution was found to be stable for at least 7 days when stored in a 25°C environment,

demonstrating superiority over the present commercial ICG formulation described in Comparative Example A. Specifically, with respect to an ICG concentration of 5 mg/ml, the 75 mg/ml inventive formulation was about 15 times more concentrated. Surprisingly, and despite the increase in concentration, the stability of the latter was about 17 times that of the former.

EXAMPLE 3

[0069] This is a further example of a preferred formulation of the present invention. This formulation is the same as that described in Example 2, except that sodium hydroxide is included with the ICG in the lyophilized vial. This change does not adversely affect the ability to provide a highly concentrated ICG solution upon reconstitution, nor the stability of that solution.

Formulation	Per ml	Total
ICG	15 mg	15 g
Sodium Hydroxide, qs pH	7.0	7.0
WFI	1 ml	1000 ml

[0070] 15 g indocyanine green is added to 800 ml water for injection and dissolved in a container. Sufficient sodium hydroxide solution is added to adjust pH to 7.0. Remaining water for injection is added to bring the solution to 1000 ml. The solution is sterilized by filtration through 0.2 micron filter. 5 ml of the sterile solution is filled into sterile vials and lyophilized. The vials are then stoppered to provide 75 mg indocyanine green per vial. The formulation is stable for a minimum of 2 years.

[0071] Sterile lyophilized 75 mg ICG vial was reconstituted with the diluent described in Example 1 to provide a 75 mg/ml ICG solution. The reconstituted solution was found to be stable for at least 7 days when stored in a 25°C environment, demonstrating superiority over the present commercial ICG formulation described in Comparative Example A. Specifically, with respect to an ICG concentration of 5 mg/ml, the 75 mg/ml inventive formulation was about 15 times more concentrated. Surprisingly, and despite the increase in concentration, the stability of the latter was about 17 times that of the former.

EXAMPLE 4

[0072] This is an example of another preferred embodiment of the present invention, a stable liposomal ICG formulation.

Formulation	Per ml	Total
ICG	2.5 mg	2.5 g
dl-alpha tocopheryl acetate	0.37 mg	0.37 g
Cholesterol	7.0 mg	7.0 g
Egg Phosphatidylcholine	23.0 mg	23.0 g
t-butyl alcohol, qs ad	1.0 ml	1000 ml

[0073] In preparing this formulation, 0.37 g dl-alpha tocopheryl acetate as dissolved in 900 ml t-butyl alcohol. 2.5 mg ICG was added thereto, and dissolved while mixing. With mixing continuing, egg phosphatidylcholine was added, followed by the addition of cholesterol. Mixing continued, with sufficient t-butyl alcohol added to bring the solution to 1000 ml. The solution was passed through a 0.2 micron filter, with 10 ml of the now sterile (filtered) solution being filled aseptically in a sterile vial. The vials were partially stoppered with a sterile stopper, and lyophilized to provide a dried cake. The lyophilizing chamber is then flushed with nitrogen to maintain an inert atmosphere, with the vials then being stoppered while in that atmosphere to maintain sterility. This liposomal (lyophilized) formulation is stable for two years. The contents of the lyophilized composition in the vial may be reconstituted with WFI or normal saline. When so reconstituted, the liposomes may be formed by gentle agitation or sonication, and remains stable for at least 24 hours.

Formulation Stability

[0074] The stability of formulations in the foregoing examples was determined by high pressure liquid chromatography (HPLC), as described below.

[0075] The equipment used to perform the analysis was a Hewlett Packard HPLC system equipped with 1050 series pumps, a 100 series variable wavelength detector and a 1050 series autosampler. The column was Supelcosil LC-18-DB, 150 x 4.66 mm, 3 um, with the analysis performed using the following gradient solution:

Time (Min)	% Solvent A	% Solvent B
0	90	10
20	20	80
25	20	80
30	90	10
35	90	10

Solvent A: 0.1% v/v Phosphoric Acid

Solvent B: Acetonitrile

[0076] Flow rate was 1 ml/min, column temperature was ambient, and detection was at 254 nm. Under these separation conditions, ICG elutes at about 14-15 min. Stability data for a 5 mg/ml ICG formulation is set forth in FIG. 3. Reconstituted ICG (at 5 mg/ml) in the inventive diluent described in Example 1 exhibited stability superior relative to ICG reconstituted in WFI at the same concentration, when stored at room temperature (25°C) and under refrigerated conditions (4-8°C).

Microbiological Stability

[0077] The bioburden for 25 mg/ml samples prepared by dissolving 25 mg lyophilized

ICG in 1 ml of the diluent of Example 1 was tested as set forth in USP.

Time	Results
0	0 cfu
7 days (25°C)	0 cfu
7 days (4-8°C)	0 cfu

Viscosity Evaluation

[0078] The viscosity of formulations provided in Comparative Example A (ICG with WFI only) was very high; a paste-like consistency. In contrast, the formulations of Examples 1 and 2, at 50 mg/ml and 75 mg/ml ICG, are easy to reconstitute and withdraw from the vial using a syringe, permitting parenteral administration to an animal.

Table I: Viscosity (cp at 25°C) of ICG Compositions Stored in a 25°C Environment

Time	25mg/ml ICG in Water	25mg ICG/ml in Example 1 Diluent	75mg/ml ICG in Water	75 mg ICG/ml in Example 1 Diluent
0	0.69	1.64	19.85	2.52
4 days	0.69	1.37	15.4	2.29
1 month	1.11	1.74	11.55	2.49

Fluorescence Evaluation

[0079] The fluorescence of the formulation in Comparative Example A was compared to that of the formulation in Example 1, using equal concentrations of ICG. Fluorescent spectra was recorded using a FluoroMax-2 (Instruments S.A., Inc.), Cuvette (1 cm lightpath), with an excitation spectra of 500-820 nm. The monchromater was set at 830 nm, slit 5 nm. The fluorescence of respective solvents was subtracted as background. The results (see FIG. 1) demonstrate excitation fluorescence that is markedly different for ICG in WFI and ICG in the diluent, the latter over about twice greater. In addition, the excitation maximum in the latter composition is slightly shifted to longer wavelengths. For emission spectra, the ICG/WFI composition was excited at 780 nm, and the ICG/diluent composition was excited at 800 nm. The emission spectra (See FIG. 2) did not show a marked difference in emission intensity. However, there was a shift in emission maxima. This shift in wavelength for emission spectra and increased excitation intensity in the inventive diluent is believed to be indicative of the difference environment surrounding the ICG molecules in solution. It may also indicate the possible interaction between ICG molecules and different components of the diluent, e.g., alcohol, PVP. These interactions are believed to stabilize the ICG, and provide for enhanced stability in an aqueous environment.

X-Ray Diffraction Analysis of Lyophilized ICG

[0080] X-ray diffraction was conducted using Cu Kα radiation from 2 to 70°2θ, an accelerating voltage of 40kV/30 mA, step size of 0.05° and an acquisition time of 2 seconds per step, with the sample spinning. FIG. 4 shows the resulting scan for the range 2 through 50° for the lyophilized material. The scan indicates that the material is amorphous and is characterized by two very broad humps centered at about 13° and 23° respectively. The lyophilized material is amorphous and hence relatively easily hydrated. The solubility of lyophilized material in WFI and the inventive diluent is comparable up to 25 mg/ml, wherein the inventive diluent is superior at concentrations above that level.

X-Ray Diffraction Analysis of Crystalline ICG

[0081] The crystalline material also has a substantial amorphous component, as observed from the broad hump of intensity in FIG 5. The pattern for this material shows, however, several peaks in the low angle region of the pattern, notably those with d-spacings of 25.8 and 17.9 Å. There is thus some degree of crystallinity observed using this analytical method. This mixed nature of non-lyophilized material results in its low solubility in WFI. The inventive diluent, however, overcomes this relatively low water solubility, and also provides the option of utilizing sterile crystalline powder for ICG-related use. The crystalline ICG is sterilized by gamma radiation or ethylene oxide sterilization techniques, with the sterile powder being filled into vials.

[0082] The inventive formulation of Example 1 is thus capable of providing enhanced therapeutic outcomes in both the diagnosis and treatment of an ailment compared to conventional ICG formulations.

EXAMPLE 5

Rat Safety Study

[0083] The ICG commercial formulation described in Comparative Example A (5 mg/ml) was tested against the formulation described in Example 2 for safety parameters. A total dose of each formulation was injected into rats at 17.5 mg/kg body weight. Thus, the volume of solution for the 5 mg/ml was 3.5 ml/kg, while for the 75 mg/ml was 0.23 ml/kg. The results of these injections are set forth below.

Table 1: Mean Body Weights (g) – Males

Group		Pretest Day 1	Dosing	Week 1	Week 2
Comparative Example A Formulation	Mean	255.4	264.8	307.6	345.2
	Std.Dev.	6.47	4.97	7.23	9.04
	(n)	(5)	(5)	(5)	(5)
Example 2 Formulation	Mean	251.2	258.4	298.6	339.0
	Std.Dev.	6.30	4.83	11.68	13.21
	(n)	(5)	(5)	(5)	(5)

Table 2: Mean Body Weights (g) – Females

Group		Pretest Day 1	Dosing	Week 1	Week 2
Comparative Example A Formulation	Mean	200.8	208.4	227.4	244.0
	Std.Dev.	8.61	8.05	9.13	13.51
	(n)	(5)	(5)	(5)	(5)
Example 2 Formulation	Mean	198.4	203.4	223.2	238.0
	Std.Dev.	126.7	6.58	5.07	8.28
	(n)	(5)	(5)	(5)	(5)

Table 3: Mean Hematology Data - Males

Group (mg/kg/day)		WBC $10^3/mm^3$	RBC $10^6/mm^3$	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	PLT $10^3/mm^3$	PT sec
Comparative Example A Formulation	Mean	8.88	6.53	14.8	40.6	62.1	22.6	36.3	948	17.8
	Std.Dev.	4.516	0.376	1.18	2.90	1.39	0.77	0.73	491.2	0.71
	(n)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Example 2 Formulation	Mean	13.3	6.55	14.7	40.0	61.1	22.5	36.9	1166	18.1
	Std.Dev.	4.637	0.194	0.45	1.82	4.31	1.21	0.70	378.6	1.12
	(n)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)

Table 4: Mean Hematology Data – Females

Group (mg/kg/day)		WBC $10^3/mm^3$	RBC $10^6/mm^3$	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	PLT $10^3/mm^3$	PT sec
Comparative Example A Formulation	Mean	10.36	6.61	14.8	42.6	64.5	22.3	34.7	1241	18.0
	Std.Dev.	0.983	0.337	0.59	1.79	1.72	0.80	0.46	117.6	0.39
	(n)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Example 2 Formulation	Mean	10.04	6.62	14.7	42.2	63.8	22.3	34.9	1020	17.7
	Std.Dev.	2.457	0.089	0.33	1.18	1.64	0.43	0.36	252.1	0.81
	(n)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)

Key to Hematology Abbreviations

Abbreviation	Parameter
Hematological Values:	
RBC	Erythrocyte Count
HGB	Hemoglobin concentration
HCT	Hematocrit
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
PLT	Platelet Count
WBC	Total Leukocyte Count
PT	Prothrombin Time

Table 5: Mean Clinical Chemistry – Males

Group		BUN mg/dL	CREAT mg/dL	ALT U/L	AST U/L	ALP U/L	T BILI mg/dL	T PRO G/dL	ALB g/dL	GLOB g/dL	AG Ratio
Comparative Example A Formulation	Mean	17.4	0.58	50.8	130.4	439.8	0.42	5.80	3.58	2.22	1.61
	Std.Dev.	0.55	0.045	3.03	12.54	106.68	0.045	0.224	0.130	0.110	0.053
	(n)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Example 2 Formulation	Mean	19.6	0.64	58.4	124.2	492.6	0.48	5.88	3.56	2.32	1.54
	Std.Dev.	2.51	0.055	13.69	6.06	199.20	0.164	0.148	0.167	0.164	0.166
	(n)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)

Table 5: Mean Clinical Chemistry – Males (Continued)

Group		GLU mg/dL	NA mEq/L	K mEq/L	CL mEq/L	CA mg/dL	I PHOS mg/dL	CREA T K U/L	CHOL mg/dL	GGT U/L	TRI-G
Comparative Example A Formulation	Mean	144.8	146.9	6.1	104.8[B]	9.9	8.2	771	66.6	0.02	133.2
	Std.Dev.	6.72	1.05	0.23	1.48	0.25	0.42	257.6	7.64	0.04	16.84
	(n)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Example 2 Formulation	Mean	146.6	146.2	5.9	106.0	10.0	8.8	581	63.4	0.10	151.8
	Std.Dev.	7.16	3.02	0.48	1.58	0.16	0.46	180.9	8.64	0.14	58.84
	(n)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)

Table 5: Mean Clinical Chemistry – Females

Group		BUN mg/dL	CREAT mg/dL	ALT U/L	AST U/L	ALP U/L	T BILI mg/dL	T PRO G/dL	ALB g/dL	GLOB g/dL	AG Ratio
Comparative Example A Formulation	Mean	19.4	0.74	49.6	122.4	260.0	0.30	6.06	3.92	2.14	1.84
	Std.Dev.	1.50	0.558	5.60	25.23	35.56	0.123	0.195	0.130	0.114	0.103
	(n)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Example 2 Formulation	Mean	19.4	0.68	58.2	123.4	257.0	0.44	5.92[A]	3.80	2.12	1.80
	Std.Dev.	1.50	0.110	12.64	24.28	44.23	0.114	0.164	0.123	0.148	0.157
	(n)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)

Table 5: Mean Clinical Chemistry – Females (Continued)

Group		GLU mg/dL	NA mEq/L	K mEq/L	CL mEq/L	CA mg/dL	I PHOS mg/dL	CREAT K U/L	CHOL mg/dL	GGT U/L	TRI-G
Comparative Example A Formulation	Mean	167.6	143.5	5.0	108.4[A]	10.2	6.8	586	69.0	0.08	76.0
	Std.Dev.	7.37	1.87	0.34	2.30	0.43	0.58	328.2	10.65	0.13	5.15
	(n)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Example 2 Formulation	Mean	174.4	146.3	4.9	108.8	10.1	7.1	512	71.2	0.12	106.4
	Std.Dev.	10.78	3.47	0.50	1.94	0.30	0.49	168.0	6.91	0.18	39.15
	(n)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)

Key to Clinical Chemistry Abbreviations

Abbreviation	Parameter	Abbreviation	Parameter
BUN	Blood Urea Nitrogen	GLU	Glucose
CREAT	Creatinine	NA	Sodium
ALT	Alanine Aminotransferase	K	Potassium
AST	Aspartate Aminotransferase	CL	Chloride
ALP	Alkaline Phosphatase	CA	Calcium
T BILI	Total Bilirubin	I PHOS	Inorganic Phosphorus
T PRO	Total Protein	CREAT K	Creatine Kinase
ALB	Albumin	CHOL	Cholesterol
GLOB	Globulin	GGT	Υ-glutamyltransferase
AG Ratio	Albumin/Globulin Ratio (calculated)		
TRI-G	Triglycerides		

[0084] The animals were observed at 1, 2.5 and 4 hours after injection, and daily for 14 days. On day 13, blood was taken for hematology and clinical chemistry determinations. Neither formulation caused any mortality nor change in blood chemistry and hematology.

[0085] The animals were necropsied on day 14 and all tissues were examined by a pathologist. The histopathology of the tissues were found to be normal.

[0086] All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference. Further, and unless otherwise indicated, references to a single component or step should be construed as also including more than one component or step, i.e., at least one. Moreover, the various components and associated numerical ranges may be included and used in the inventive compositions independent of one another and also of the other components.

[0087] While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.